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# **The Spinning Cloth Disc Reactor for Immobilized Enzymes: A New Process Intensification Technology for Enzymatic Reactions**

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## Abstract

The Spinning Cloth Disc Reactor (SCDR) is an innovative enzyme reaction intensification technology. Based on spinning disc technology, the SCDR uses centrifugal forces to allow an even spread of a thin film across a spinning horizontal disc which holds a cloth with immobilized enzyme. This geometry promotes accelerated reactions due to high mass transfer rates and rapid mixing. Here, the SCDR has been benchmarked against a conventional Batch Stirred Tank Reactor (BSTR) using tributyrin emulsion hydrolysis as a model reaction and lipase immobilized on woolen cloth as the biocatalyst. Reaction intensification has been shown to occur: the conversion in the SCDR was significantly higher than that in a conventional BSTR under comparable conditions. Spinning speed and flow rate control reaction rate and conversion: conversion increased nearly 7% on average as the flow rate rose from 2 to 5 mL s<sup>-1</sup> and the highest conversion (72.1%) occurred at 400 rpm. A Ping Pong Bi Bi kinetic model fitted reaction progress data well. The immobilized lipase showed excellent stability to repeat reactions in the SCDR: 80% of the original activity was retained after 15 consecutive runs. The robustness of the SCDR to industrially relevant feeds was also demonstrated through successful hydrolysis of different vegetable oils at reaction rates 5 times higher than other reactors in the literature. Overall, the above results indicate that the SCDR is an innovative, superior and robust technology for enhancing enzyme reactions, taking enzyme reactors beyond the current state-of-the-art. This concept can readily be extended to other enzyme-catalyzed reactions, where enhanced mass transfer and enzyme stability is needed.

*Keywords:* spinning cloth disc reactor; lipase immobilization; woolen cloth support; oil hydrolysis; enzyme reaction intensification.

## 1. Introduction

Enzymatic hydrolysis of triglycerides into acids by lipase is an environmentally sustainable alternative to chemical hydrolysis and can be used in many important industrial applications (such as in the fat and oleochemical industry, the dairy industry and wastewater treatment), due to the potential energy savings and alleviation of thermal deactivation of unsaturated fatty acids through lowering the reaction temperature [1, 2]. One significant characteristic of lipase in this reaction is its activation at the oil-water interface; therefore such hydrolysis reactions catalyzed by lipase are more effective in oil/water emulsions [3]. In practical applications, immobilized lipase is more favorable due to the prominent advantages over its free form: enhanced stability, ease of enzyme recovery and reuse, simplified product separation. Thus, immobilized enzyme reactors have been widely studied for industrial processes [4]. Batch stirred tank reactors (BSTR) are the most commonly used reactors for enzymatic processes, however this type of reactor suffers from a series of disadvantages, including the fact that active enzymes (in their free form) are complicated to recover and reuse and low productivities [5]. Furthermore, mass transfer can be limiting, with only increased stirring speed as the means of reducing the mass transfer resistances inherent in any enzyme immobilization and support used. Consequently, a range of enzyme reactors have been proposed for lipase catalyzed reactions to overcome such disadvantages. For example:

- Packed bed reactors (PBRs), which offer great advantages for immobilized enzymes, such as high efficiency, low cost and ease of construction. PBRs with immobilized lipase have been used for rice bran oil hydrolysis [6, 7]. However, the main drawbacks of PBRs are the associated large pressure drops (if the packing is too small) as well as potential bypassing and channeling if the catalyst is improperly packed [8]. To produce a low pressure drop, large particles are required; however this decreases the amount of enzyme per volume in the reactor (decreasing overall reactor efficiency).

1 • Fluidized bed reactors (FBRs) have been reported for oil hydrolysis with immobilized  
2 lipase [9]. One of the potential advantages of FBRs are that small particles can be used  
3 (since pressure drop is unaffected), however large particles are usually required anyway  
4 due to the low density difference between fluid and particles used in immobilized enzyme  
5 FBR systems, and the high viscosity of the fluids usually used. This again decreases the  
6 amount of enzyme per volume in the reactor, decreasing overall reactor efficiency.  
7 Moreover, significant channeling and bypassing of the particles as well as significant  
8 particle agitation (and therefore the potential for enzyme loss and deactivation) also make  
9 FBRs complicated to operate for enzymatic reactions.

10 • Enzyme membrane reactors (EMRs) have become increasingly popular due to their  
11 integration of enzyme catalyzed conversion and product separation into a single process.  
12 EMRs have been widely used with immobilized lipase for triglyceride hydrolysis [10-12].  
13 However, EMRs typically undergo a decrease in reaction rate and yield during operation  
14 caused by loss of catalyst and mass transfer efficiency (including the effects of membrane  
15 fouling), limiting their industrial application.

16 Based on this, it is clear that a robust and stable enzyme reactor that maintains a stable  
17 immobilized enzyme at a high amount of enzyme per volume in the reactor combined with  
18 intensified mass transfer is very desirable. Therefore, this study investigates the application of  
19 process intensification to immobilized enzyme reactors, applying the concept of the spinning  
20 disc reactor (SDR) for the first time to immobilized enzyme systems.

21 Process intensification has been recognized as a promising development path for the chemical  
22 process industry, aiming to improve production efficiency, lower cost, enhance safety and  
23 reduce environmental pollution [13, 14]. The SDR is one such technology, which consists of  
24 a rotating disc with a jet of liquid impinging onto the center of its top surface. The centrifugal  
25 force of the spinning disc forces this liquid to form a thin (100 to 200  $\mu\text{m}$ ) and highly sheared

1 film on top of the rotating surface. Research has shown that the heat and mass transfer in such  
2 device can be significantly enhanced by the fluid dynamics within these films [15-18]. In  
3 addition, the SDR also has several benefits over conventional reactors, such as the rapid  
4 mixing in the liquid film and short liquid residence times [19]. Due to these factors, the SDR  
5 has been used to enhance reaction rates in a range of chemical reactions including:  
6 condensation polymerizations [20], nanoparticle preparation [21-24], biodiesel synthesis[25],  
7 pharmaceutical manufacture [26], and thin film photocatalysis [27]. Despite the wide range of  
8 reactions that SDRs have been applied to, to authors' best knowledge, the SDR concept has  
9 not yet been applied to enzyme reactions nor to catalyst systems immobilized within a three  
10 dimensional mesh, such as a fibrous cloth. Recently, a simple and effective protocol has been  
11 developed by the authors to immobilize lipase on woolen cloth with high enzyme load,  
12 activity and good reusability [28]. The protocol consists of four main steps: (1) Bleaching of  
13 the wool surface to release viable functional groups for immobilization, (2) modification of  
14 the bleached wool surface with polyethyleneimine, (3) adsorption of lipase onto the  
15 polyethyleneimine modified wool by electrostatic interaction, and (4) cross-linking with  
16 glutaraldehyde to stabilize the immobilized lipase. Confocal laser scanning microscopy was  
17 used to confirm that immobilization occurred both on the outer surface and within the volume  
18 of cloth. This provides a large outer and inner fiber surface area facilitating contact and  
19 reaction of substrates with the immobilized enzymes [28]. This immobilized lipase on wool  
20 therefore has great potential as a matrix for immobilized enzymes in a spinning disc reactor  
21 system.

22 Therefore, through combining the SDR concept with this superior woolen cloth enzyme  
23 immobilization, this paper presents both a new method of process intensification in  
24 enzymatic reactions as well as a novel type of rotating reactor system: the spinning cloth disc  
25 reactor (SCDR). Based on the principles of the SDR, the SCDR also uses centrifugal forces

to allow a spread of a thin film across a spinning horizontal disc; however this disc has a cloth with immobilized enzyme. The SCDR therefore potentially produces a thin liquid film flow both on top of and through the cloth. The cloth surface is key to increasing the potential of immobilized enzymes in a variety of reactions, since it should produce accelerated reaction rates due to high mass transfer rates and rapid mixing on top and within the cloth, with the cloth potentially helping protect the enzyme from excessive hydrodynamic forces, as well as providing an additional structure that can promote mixing and turbulence at the appropriate spinning speeds and feed flow rates. As such, the purpose of this research is to prove the viability of the SCDR concept and characterize its performance, using immobilized lipase onto woolen cloth with tributyrin emulsion hydrolysis as the model reaction. The SCDR will be benchmarked against a conventional BSTR run under equivalent conditions to determine if enzyme process intensification is achieved (and therefore if the SCDR is a worthwhile technology to pursue).

## **2. Materials and Methods**

### *2.1. Materials*

Unbleached organic woolen cloth (color: natural cream, thickness: 1.5 mm) was purchased from Treliske (Otago, New Zealand). Amano lipase derived from *Pseudomonas fluorescens*, polyethyleneimine (PEI; branched, average MW of 10,000), tributyrin (98%), tritonX-100, coomassie brilliant blue G 250, sodium bicarbonate and sodium carbonate were obtained from Sigma-Aldrich (New Zealand). Glutaraldehyde (GA) 25% (w/v), sodium dihydrogen phosphate, disodium hydrogen phosphate and hydrochloric acid were purchased from Unilab (ECP, New Zealand). Hydrogen peroxide 30% (v/v) was obtained from Scharlau (Thermofisher, New Zealand). Bovine serum albumin was obtained from Gibcobl (Life Technologies, New Zealand). Canola oil, olive oil, soybean oil and sunflower oil were obtained from the local market. The phosphate buffer (pH 6 and pH 7) used in this study was

composed of 0.1M sodium dihydrogen phosphate and disodium hydrogen phosphate. All solutions were prepared using deionized water (produced from Milli-Q Gradient A10 made by Millipore).

## *2.2. Immobilization of lipase on woolen cloth*

The main immobilization procedure has been described in detail elsewhere [28]. The woolen cloth was cut into circular pieces with a diameter of 250 mm, weighing 16 g. First, the woolen cloth was pretreated with a solution of 30 mL L<sup>-1</sup> hydrogen peroxide (30%) and 2 g L<sup>-1</sup> sodium silicate at pH 9 (0.1 M Na<sub>2</sub>CO<sub>3</sub>, NaHCO<sub>3</sub> buffer) at 55 °C for 70 min. The bleached woolen cloth was then dipped in 500 mL 2% PEI solution at pH 8 (adjusted with hydrochloric acid) for 2 h at room temperature and rinsed with deionized water. The cloth was thereafter soaked in 1 L 2 mg mL<sup>-1</sup> lipase solution (0.1 M Na<sub>2</sub>HPO<sub>4</sub>, NaH<sub>2</sub>PO<sub>4</sub> buffer, pH 6) for 24 h, followed by immersion in 500 mL 0.5% (w/v) GA solution (0.1 M phosphate buffer, pH 6) for 10 min for crosslinking. The cloth was finally washed with deionized water until no free enzyme was detected in the washed solution using the Bradford assay [29]. The enzyme load was 46.8 mg per dry gram of cloth determined by measuring the protein content of the enzyme solution with the Bradford method before and after immobilization [29]. The enzyme activity was 178.3 U per dry gram of woolen cloth, using the tributyrin emulsion hydrolysis method previously described by the authors [28]. One enzyme unit (U) was defined as the amount of lipase which catalyzes the release of 1 μmol butyric acid per minute under the tested conditions.

## *2.3. Tributyrin hydrolysis in the SCDR*

All experiments were repeated at least three times and results are presented as the average ± one standard deviation.



A schematic diagram and photos of the circulating batch SCDR process used in this study are shown in Fig. 1. The SCDR is an overhead, centrally fed system. It has an overhead stirrer with a variable speed motor (Glas-Gol, 399132, US) connected by a metal rod to a Perspex disc 250 mm in diameter – this disc is the critical spinning surface that the woolen cloth rests on. The woolen cloth with immobilized lipase was rested (with no means of fastening) on the disc as shown in Fig. 1c. A steel funnel-shaped chamber (300 mm in diameter and 210 mm deep) surrounds the disc. The reaction system consists of two loops from the reactant/product vessel. The first loop is for the reaction: liquid reactants are pumped into the reactor with a peristaltic pump (Cole-Parmer, 7553-75, US) to a liquid feed pipe at the center of the spinning disc. The feed pipe is positioned so that the liquid feed impacts on the wool with minimal splashing and produces maximum wetting of the woolen cloth. As a result, the solution was spread over spinning cloth surface and within the volume of the cloth by the centrifugal force, allowing the tributyrin to contact and be hydrolyzed by the immobilized lipase on the disc. The reactor funnel contains the liquid spun off the edge of the disc and cloth (so it does not splash out of the reactor system) so that this liquid can be funneled down to the pipes that feed it back to the reactant/product vessel. The second loop consists of a pH stat (Metrohm, Switzerland), where during a reaction, sodium hydroxide was added into the reactant vessel from the pH stat to keep a constant pH and the data was collected continuously with a PC via the Tiamo 1.3 program (Metrohm, Switzerland). Reaction conversion was correlated to moles of sodium hydroxide consumed by the reaction according to Eq. (1):

$$Conversion(\%) = \frac{\text{moles of free butyric acids}}{\text{moles of original esters in tributyrin}} \times 100 \quad (1)$$

To ensure the accuracy of the pH stat, conversion of tributyrin was also verified using samples collected throughout the course of the reaction that were analyzed by gas

chromatography (GC) - see Supplementary Material A for details and full results. In summary: the pH stat and GC results were in good agreement, demonstrating that the kinetic data from pH stat can be used to evaluate the reaction kinetics.

In a typical reaction, the tributyrin emulsion was prepared by adding tributyrin and triton X-100 to the desired volume of phosphate buffer (0.1 M, pH 7) with a final concentration of 33 mM and 3.5 g L<sup>-1</sup>, respectively. The mixture was then emulsified with a motor homogenizer (IKA T25 digital, Japan) at 12,000 rpm for 5 min. To start up the reactor, the disc with immobilized lipase woolen cloth was firstly connected to the driving motor and spun to the desired rotational speed. After that, the reaction was initiated by starting the pump so that the tributyrin emulsion was fed to the center of the spinning disc and the system was left to run for 4 h. Reaction data was continuously collected by the pH stat system. The effect of flow rate, spinning speed and surface shear on reaction rate and loss of enzyme from the reactor was studied. The surface shear on the spinning disc increased with the increase of radial distance and reached the maximum value on the disc edge. The surface shear was calculated with the following equation [30]:

$$S = \left( \frac{3Qr\omega^4}{2\pi\nu^2} \right)^{1/3} \quad (2)$$

Where:  $S$  = surface shear (s<sup>-1</sup>);  $Q$  = volumetric flow rate (m<sup>3</sup> s<sup>-1</sup>);  $r$  = radial distance (m);

$\Omega$  = angular velocity (rad s<sup>-1</sup>);  $\nu$  = kinematic viscosity (m<sup>2</sup> s<sup>-1</sup>).

Using the equation for the distribution of surface shear on the disc, the average surface shear can be calculated as:

$$\bar{S} = \frac{1}{R} \int_0^R S dr = \frac{3}{4} \left( \frac{3QR\omega^4}{2\pi\nu^2} \right)^{1/3} \quad (3)$$

Where:  $R$  = the radius of the disc.

1 In this study, the average surface shear was used to characterize the performance of SCDR.  
2 Control runs were also carried out in the SCDR without immobilized lipase and no reaction  
3 took place.

4 The reusability of the immobilized lipase on wool in the SCDR was determined by measuring  
5 the activity of the same cloth in tributyrin hydrolysis over a number of cycles, with a reaction  
6 temperature of 45 °C; reaction time of 4 h, feed flow rate of 5 mL s<sup>-1</sup> and a spinning speed of  
7 350 rpm (average shear = 8,600 s<sup>-1</sup>). After each consecutive run, the woolen cloth was  
8 washed with phosphate buffer (pH 7, 0.1 M) and the previous reaction solution was replaced  
9 with a fresh solution. Three different cloths were evaluated in order to determine the  
10 repeatability; the original activity of the first batch was taken to be 100%. The leakage (i.e.  
11 detachment of enzyme from the cloth) of immobilized enzyme was also studied in terms of  
12 the average surface shear. In these experiments, the amount of enzyme lost from the woolen  
13 support was determined by measuring the amount of free enzyme in the reaction solution by  
14 detecting its UV absorbance at 280 nm. Since the tributyrin emulsion would interfere with  
15 this measurement, only 0.1M phosphate buffer (pH 7) was used as feed for the SCDR, with  
16 all other operational variables kept the same as for the tributyrin hydrolysis.

17 To determine if solution was able to penetrate in order to contact all of the immobilized lipase  
18 in the woolen cloth, the spinning cloth with and without immobilized lipase was fed a dye  
19 solution (water color, Reeves, UK) until the cloth was saturated. Taking the cloth off the  
20 reactor it was observed that both sides of both cloths (with and without immobilized lipase)  
21 presented the color to the same extent, indicating that there was also flow within the cloth in  
22 SCDR.

#### 23 2.4. Tributyrin hydrolysis in the BSTR

In order to compare the results obtained from the SCDR, tributyrin hydrolysis with both free and immobilized lipase was performed in a batch reactor. The tributyrin emulsion was prepared the same way as in the SCDR experiments. The reaction was started by adding the same amount of free or immobilized lipase on woolen cloth to the substrate. The pH stat was applied to monitor the reaction and maintain a constant pH. Experiments to compare the BSTR and SCDR were made under the same reaction conditions: temperature of 45 °C and substrate concentration of 33 mM in 0.1 M pH 7 phosphate buffer. The same enzyme to substrate ratio was maintained in comparing the SCDR and BSTR.

### *2.5. Tributyrin emulsion droplet size measurements*

The effect of the spinning cloth on the emulsion droplet size was also determined. A cloth without immobilized lipase was used in this section in order to isolate the effect of the cloth from the effect of the reaction on emulsion droplet size. The tributyrin emulsion (66 mM), was prepared as described in Section 2.3 and was fed to the spinning disc with and without cloth at flow rate of 5 mL s<sup>-1</sup> and spinning speed of 450 rpm. Samples were taken periodically and the particle size (d<sub>0.5</sub>) was measured with a Particle Size Analyzer (Malvern Mastersizer 2000, UK).

### *2.6. Kinetic analysis*

It has been established that the hydrolysis of triglycerides is usually well described by a Ping Pong Bi Bi mechanism [31]. According to this model, reactions take place in a multistep process where one product is released and then another substrate combines with the enzyme [32]. In this study, a Ping Pong Bi Bi mechanism with a rate controlling step of deacylation was used for acquiring kinetic parameters in this study (see Supplementary Material B). These kinetics were fitted over the entire reaction period, to provide ‘reaction progress kinetics’, which provide a description of the reaction over the entire reactor operation rather

than just the initial stages as the more commonly used initial rate kinetics gives. This approach better represents the reaction, since enzyme reaction rates are more often than not affected by factors that occur after the initial rate period. The rate expression is as follows [31]:

$$v = \frac{(v_{\max} / K_m) S}{1 + S / K_m + (S_0 - S) / K_i} \quad (4)$$

Where  $v_{\max}$ ,  $K_m$  and  $K_i$  are lumped ping pong kinetic constants, and their expressions are listed in Supplementary Material B;  $S_0$  is the initial concentration of the glyceride. Since  $K_m$  is typically far larger than  $S$  at low substrate concentration [31], Eq. (4) can be further simplified as follows:

$$v = \frac{(v_{\max} / K_m) S}{1 + (S_0 - S) / K_i} \quad (5)$$

Eq. (5) can be integrated to give a relationship of  $x$  (conversion) versus  $t$ :

$$-\frac{S_0}{K_i} x - \left( \frac{S_0}{K_i} + 1 \right) \ln(1 - x) = \frac{v_{\max}}{K_m} t \quad (6)$$

Application of a non-linear regression shows that  $v_{\max}$  and  $K_m$  cannot be determined at the same time, hence  $v_{\max}/K_m$  was estimated as one parameter and used to denote the hydrolyzing efficiency of the enzyme. In order to derive this parameter, experiments were performed at various initial tributyrin concentrations (33, 66, 99, 132 mM) and the continuous data over the entire reaction period from the pH stat was collected to determine the constants in Eq. (6) with the curve fitting tool in MATLAB.

### 3. Results and discussion

#### 3.1. Is the SCDR a true enzyme process intensification technology?

Fig. 2 (a-d) shows a comparison of tributyrin conversion at different initial concentrations (from 33 to 132 mM) between the SCDR and the BSTR with immobilized and free lipase. Fig. 2e shows the initial rates (taken for the first 30 min). Overall, Fig. 2(a-e) shows that the SCDR with immobilized lipase is the optimal operational mode and that process intensification occurs in the SCDR, indicating that the SCDR is a worthwhile technology to further pursue. In particular, Fig. 2 (a-d) shows that, under comparable reaction conditions, tributyrin hydrolysis at all investigated concentrations has a higher conversion in the SCDR than in the BSTR for both immobilized and free lipase. The improved reaction conversion in the SCDR is most likely attributable the expected more rapid mixing of substrate to the immobilized lipase and reduced mass transfer resistances between the enzyme and substrate in the thin film on top of and within the cloth compared to that in the BSTR, caused by the centrifugally forced liquid flow over the immobilized enzymes. This is further confirmed by Fig 2a that shows that when free lipase (in the same amount as the enzyme load on cloth) was used in the SCDR without cloth, the conversion was increased 9.4% in comparison to that in the BSTR. Thus the expected enhanced mass transfer of spinning disc technology is able to increase the reaction rates of free lipase also.

The SCDR maintained a similar reaction rate in the first reaction stage to the free lipase in the BSTR (Fig. 2e). This again implies that the mass transfer limitations of immobilized lipase are significantly overcome in SCDR, confirming that it is enhanced mass transfer causing the process intensification. Moreover, Fig. 2a also shows that immobilized enzymes rather than free enzymes are optimal in the SCDR. The conversion of free lipase in SCDR was 7.2% lower than that with immobilized lipase. This is unexpected, since often immobilised enzymes have a lower activity compared to free enzymes - there can be the activity loss due

1 to deactivation from the chemicals used and the steric effects during the immobilization  
2 process [24]. Furthermore, there are inherent mass transfer limitations during reactions of  
3 immobilized enzymes compared to their free forms. Here this is mainly because the lipase is  
4 immobilized both on the outer surface and inside the cloth. For the tributyrin in the bulk  
5 solution contacting the cloth to reach these enzymes, there are significant mass transfer  
6 resistances compared to the enzyme in the free form: the tributyrin needs to mass transfer  
7 from the bulk solution to the immobilized lipases on the outer surface (through a stagnant  
8 film layer) and some need to then mass transfer into the cloth to the enzymes on the inner  
9 wool fiber surfaces. This is why in the BSTR, free lipase shows the expected higher  
10 conversion and reaction rate than immobilized lipase. This indicates that more than mass  
11 transfer resistances are being overcome by the SCDR to increase the reaction rate and yield  
12 of the wool immobilised enzymes in the SCDR. It is hypothesized that the combination of  
13 cloth matrix and centrifugally forced liquid is responsible in two different ways: (1) oil layers  
14 on the high surface area of woolen cloth and/or by this woolen cloth helping to produce and  
15 maintain the oil water emulsion through sieving and fracturing of the oil is helping to  
16 maintain a sufficient interfacial area for high enzyme activity for reaction between the lipase  
17 and the oil throughout the reaction; and (2) the longer residence time of the substrate in the  
18 cloth as well as the fact that the enzymes, when immobilized, are in contact with the  
19 tributyrin substrate in the reaction intensified zone for a longer period, since they constantly  
20 reside on the spinning disc, unlike the free enzymes – they have a discrete residence time on  
21 the spinning disk. Both of these points will therefore be explored further in this paper –  
22 interfacial surface area below and residence time in Section 3.2.

23 In terms of enhancing and maintain interfacial area for reaction, the fact that there is a lower  
24 reaction rate in the BSTR in the second part of the reaction compared to the SCDR (Fig. 2)  
25 provides evidence that the emulsion and associated higher interfacial surface area which is

required for high lipase activity (as enzymes are interfacially activated [3]) is maintained by the SCDR. To further investigate the effect of the cloth on the emulsion droplet size, the droplet size was evaluated in the SCDR with and without a cloth, with no enzymes presents (i.e. no reaction). Figure 3 shows that without the cloth, there was no significant decrease in the droplet size however, in comparison, with the cloth present, a significant decrease was observed (3.5 to 1.1  $\mu\text{m}$  after 4h). As there was no reaction, this is likely due to a sieving action of the cloth as the emulsion flows on the surface/ within the cloth. This shows that process intensification in the SCDR is also produced by interfacial area maintenance/enhancement – a unique result for spinning disc reactor technology and enzyme process intensification.

Finally, the volumetric loading of the SCDR and BSTR were compared: to explore the potential of increasing the volume of feed that can be processed with the same amount of immobilized lipase and same size disc in this SCDR, 2 L feed of the same concentration was run through the SCDR. As shown in Fig. 2a, the conversion was 60.3% (decreased by 12%), yet higher than in the BSTR with both free and immobilized lipases, even when the feed was doubled to 2 L, further underlining the high efficiency and catalytic capacity of the SCDR. For BSTRs, changing the reaction volume affects the mixing and turbulence inside the reactor, thus affect the reaction rate. However, for the SCDR the reaction volume only increases the amount of substrate, because the reaction does not occur in the feed vessel. Therefore, this shows that it is easier to increase the volumetric loading of a single SCDR than in a BSTR.

### *3.2. Optimizing the SCDR: the effect of feed flow rate and disc speed on conversion*

The average shear ( $\bar{S}$  ; Eq. 3) and mean residence time are two of the most important



variables for spinning disc technology, since the average shear rate denotes the degree of mixing obtained in the reacting film and the mean residence time represents the contact time between the reactants on the disc [33]. In this study, these two parameters were adjusted by changing the feed flow rate and disc speed and their effect on tributyrin emulsion hydrolysis was investigated.

As can be seen from Fig. 4, the conversion increased approximately 7% on average as the flow rate increased from 2 to 5 mL s<sup>-1</sup>. A higher flow rate increases the contact frequency (i.e. there are more passes of a reactant molecule to the spinning disc in the system at a higher flow rate), but decreases the mean residence time and therefore contact time between reactants and lipase on the disc per pass. In terms of conversion, these two factors act against each other: the decreased residence time per pass decreases conversion and the increased contact frequency increases conversion. Since conversion increased 7% despite the fact that the increase of flow rate reduced the mean residence time per pass by 48% (determined from residence time distribution (RTD) on the spinning cloth disc with a pulse injection technique), the residence time is not the important factor. Increased contact frequency would partly explain this, however at higher flow rates, one would expect that mixing and mass transfer resistances to be reduced as well as there being an increased hydrodynamic force pushing oil droplets through the mesh structure of the cloth (and decreasing droplet size as a result - further enhancing the trend shown in Fig. 3). More liquid would also be forced into the woolen cloth, providing greater reactant contact with the immobilized enzymes within. These factors would all contribute to increased conversion.

For a flow rate of 5 mL s<sup>-1</sup>, as the disc speed increased from 250 to 400 rpm, the conversion increased from 67.7% to 72.1%. This is likely due to enhanced mixing and reduction in mass transfer resistances around the immobilized enzymes on top of and within the woolen cloth. The increased centrifugal force may also have forced more liquid into the woolen cloth,

providing greater reactant contact with the immobilized enzymes. However, the conversion decreased to 69.2% with a further increase of disc speed to 500 rpm. This most likely can be attributed to two factors:

(1). The decreased liquid holdup and residence time decreasing reaction effectiveness. Residence time distribution on the spinning cloth disc has been studied with a pulse injection technique and it was found that the residence time decreased with an increase in disc speed. This data will not be presented here due to space restrictions, but will be presented in full in a future publication.

(2) The average shear rate increased from  $10,200\text{ s}^{-1}$  to  $13,800\text{ s}^{-1}$  with a rise in disc spinning speed from 400 to 500 rpm for a flow rate of  $5\text{ mL s}^{-1}$  (see Supplementary Material C). Therefore it is possible that the higher shear force would either deactivate the enzymes or cause enzyme detachment from the cloth. This will be further investigated in Section 3.3.

The fact that higher spinning speed is detrimental to the reaction is a significantly different phenomena compared to many other systems and reactions in more conventional SDRs, where the general trend is that a higher spinning speed produces an increase in either reaction rate or optimization of the desired product. For example, when spinning disc technology was used to synthesize nanoparticles, a disc speed of 1000 to 4000 rpm was used to increase localized mixing, turbulence and shear to obtain smaller nanoparticle sizes [21, 22, 34, 35]. The one comparable application is the photocatalytic degradation of methylene blue in SDR [27], where the optimum feed flow rate and spinning speed were determined to be  $15\text{ mL s}^{-1}$  and 100 to 200 rpm due to favorable wave patterns and mixing at these conditions reducing the light penetration depth and mass transfer resistance on the thin film. The optimum in this case is caused by different factors though: a relatively low disc speed is required when the spinning disc technology is applied to cloth immobilized enzymes to optimize liquid holdup and residence time with mass transfer advantages and to ensure the shear on the enzymes is

below the threshold that causes deactivation and enzyme detachment from the support. This critical shear threshold must therefore be quantified.

### *3.3. Loss of immobilized lipase from the woolen support in the SCDR*

As discussed above, the high surface shear produced by the SCDR can lead to an enhanced reaction rate, but also increase the detachment of the enzyme from the spinning cloth support. Therefore, the leakage of immobilized enzyme was studied in terms of the average surface shear. Fig. 5 presents the amount of enzyme lost from the support as a function of average surface shear in the SCDR. As expected, the loss of enzyme increased with increasing surface shear; up to a maximum of 7 mg over a 4 h run. This however only amounts to 0.9% of the entire enzyme on the cloth per run, indicating the immobilized lipase is strongly supported onto the wool, even under a relatively high shear force. Fig. 5 also shows that there are two loss regimes. Up to an average surface shear of  $9,500 \text{ s}^{-1}$  there is a gradual increase in enzyme loss with a gradient of  $0.0002 \text{ mg s}$  if assuming a linear trend. After this, there is a critical shear at which a higher loss of enzyme is produced. This indicates that the cloth is protecting the enzymes from the high shear environment on the disc, at least up to the critical average shear threshold of  $9,500 \text{ s}^{-1}$ .

An explanation of why there are two loss regions can be made if the flow on the disc is considered as having two distinct regions: (I) the flow on the top outer surface of the cloth, and (II) the flow through the inner bulk of the cloth. This critical shear threshold should then be interpreted in terms of these two regions. Region I would have relatively little resistance to fluid flow, since it flows across a free surface and therefore would have a higher localized fluid velocity and shear force than region II, which due to the resistances to flow caused by the fibers within the bulk cloth would have a lower overall volumetric flow rate and localized

1 velocity and consequently would have a lower localized shear force. Therefore, the first  
2 lower rate of loss could be interpreted as a loss of the more loosely bound enzymes from the  
3 outer surface of the cloth. As the shear force increases, more liquid was forced into the cloth  
4 from the top surface and gradually detach more of the loosely bound enzymes (as well as  
5 more enzymes from the outer surface). After the critical average shear of  $9,500\text{ s}^{-1}$ , there may  
6 be sufficient hydrodynamic force at and near the outer cloth surface to completely detach the  
7 enzymes from the wool fibers and therefore the loss rate of enzymes from this critical shear  
8 threshold increases.

9 Therefore, in view of the stability of immobilized enzymes, the SCDR should be operated  
10 below an average surface shear of  $9,500\text{ s}^{-1}$ . This value is a bit lower than the previously  
11 determined optimal spinning speed of 400 rpm for a flow rate of  $5\text{ mL s}^{-1}$  (around  $10,300\text{ s}^{-1}$ ).  
12 This may be due to a slight difference in fluid interactions in the absence of tributyrin and  
13 reaction in these experiments. However, in order to preserve a margin of safety, a lower  
14 spinning speed of 350 rpm was employed in all further experiments. This also indicates that  
15 to further improve the SCDR, especially at higher shear rates, further work on improving the  
16 stability of immobilized lipase could to be done either by looking at other support materials  
17 or some surface modification to more strongly bind the lipase to the wool.

#### 19 *3.4. Stability and reusability of wool immobilized lipase in the SCDR*

20 As shown in Fig. 6, the immobilized lipase exhibited good reusability over many cycles,  
21 maintaining around 80% activity after being used 15 consecutive times in SCDR. This  
22 illustrates that this system, and the SCDR in particular, produces not only an intensified  
23 reaction, but is stable over multiple cycles, indicating that it is a very promising candidate as  
24 a stable industrial immobilized enzyme reactor.

The enzyme activity loss can be attributed to two factors: (1) loss of enzyme from the spinning cloth; (2) deactivation of the immobilized enzymes (which may be due to shear based deactivation as well as substrate, intermediates, product and/or reaction related inhibition/deactivation). The former is because it was shown that detached enzymes do cause a loss in activity. Additional experiments (see Supplementary Material D) demonstrated that the small amount of leaked enzymes during each run did not show significant activity and had insignificant effect on the reaction kinetics and yield. Experiments have also shown that product inhibition is most likely negligible (see Supplementary Material E).

To further clarify the relative importance of these factors in the relationship between leakage of enzyme and activity loss, the enzyme leakage in a further 15 continuous runs was studied. As can be seen from Fig. 6, the loss of enzyme from the woolen support decreased with repeated use of the same cloth, indicating that only the loosely bound lipase was lost, and the left lipase was well attached after repeated reactions in the SCDR over many cycles. It should be noted that this enzyme loss was measured with phosphate buffer; however this value may change when the immobilized lipase is used with the tributyrin present. However, since the detached enzymes cannot be measured with the tributyrin, the enzyme loss with only phosphate buffer is used and the results should be interpreted with this limitation in mind. Therefore, the leakage results in Fig. 6 can be interpreted as follows: in the first three runs, the loss of enzyme from the support declined fast. This is most likely due to the loss of those enzymes that were not covalently attached to the woolen cloth support, either by incomplete reaction with the PEI, incomplete crosslinking by GA and those that were only absorbed on the surfaces of the woolen cloth. After 3 runs, the enzyme leakage became relatively stable (from 4 to 7 runs) and then decreased again, and after 15 runs, the leakage of enzyme was 1.9 mg (36.5% of the first run), very small considering the total enzyme immobilized on woolen cloth was 748 mg. After 15 runs, the total amount of enzyme leakage was 53.6 mg. After the

1 15 consecutive runs with phosphate buffer, the remaining activity of the cloth was evaluated  
2 to be 90.5%. The activity loss for this system cannot be due to reactant/reaction  
3 intermediate/reaction based inhibition/deactivation, therefore since the total enzyme leakage  
4 of 53.6 mg is equivalent to 7.2% of the total activity and the total activity loss was 9.5%, the  
5 activity loss by shear force deactivation was therefore the difference - only 2.3%. This means  
6 that enzyme leakage is the major cause of enzyme deactivation in the absence of reaction. But  
7 with a further increase in shear force, the deactivation by the shear force may be expected to  
8 further increase. This result substantiates the hypothesis in Section 3.2 that the reduction in  
9 conversion can be partially attributed to the activity loss by high shear forces.

10 When considering the 15 consecutive runs in the tributyrin reacting system, since the total  
11 activity loss after 15 reactions was 20.3% and enzyme leakage was 7.2%, deactivation by  
12 shear force was 2.3%, the remaining 10.8% activity loss can be attributed to deactivation of  
13 the attached enzymes by the substrate, intermediates and/or the reaction. Note that there may  
14 also have been further enzyme detachment caused by tributyrin (which was not present in the  
15 enzyme leakage experiments), but this cannot be quantified with the methods used. Therefore,  
16 within the stated conditions of the experiments, these results show that deactivation  
17 contributed roughly equally to the relatively small activity loss in the SCDR over 15 cycles.  
18 This indicates that the woolen cloth is an effective support and lipase immobilized system for  
19 use in the SCDR, since it protects a majority of the enzymes from shear. This is in agreement  
20 with the results in Fig. 2(a-e), where it shows that the immobilized enzymes retain a higher  
21 activity than the free enzymes.

### 22 23 3.5. Kinetics of tributyrin hydrolysis in the SCDR

In order to apply the SCDR at an industrial scale, the reaction profile and kinetics need to be quantified. Fig. 7a shows the conversion of tributyrin with time in the SCDR at various initial tributyrin concentrations. The effect of increasing the substrate concentration is clear: the conversion decreased from 72% to 49% as the substrate concentration increased from 33 to 132 mM. In addition, as also observed in Section 3.1 (and Figure 2), the tributyrin hydrolysis has two reaction stages for all of the different concentrations used. This is most clearly seen in terms of reaction progress rate versus concentration (Fig. 7b). Firstly, the reaction rate was relatively fast until approximately 20% to 25% conversion was achieved and it decreased with a similar slope for all different concentrations. Literature indicates that this can be attributed to a decrease in droplet size during this reaction [36],[37]. Product inhibition by fatty acids and monoglyceride [3, 38] may play a part, but it is likely to be insignificant in this study due to the short chain of tributyrin (and further experiments have also confirmed this - see Supplementary Material E). In addition, it can visually be observed that the reaction solution gradually turned from cloudy white to clear as the reaction proceeded. It has been reported that TritonX-100 and lipid/triglyceride can form pure and mixed micelles in aqueous system, and such micelles are optically clear [39-41]. When pure surfactant micelles, mixed micelles, and oil droplets coexist, the largest portion of oil (>95%) is dispersed as oil droplets [42, 43]. As the hydrolysis proceeds, the number of oil droplets decreases and the products are more hydrophilic, and the small amount of remaining diglyceride and triglyceride may exist in the form of micelles, so the solution gradually becomes clear. Note that although there is a rate reduction, it is not as significant as in other reactors under equivalent reaction conditions, as discussed in Section 3.1 and shown in Fig. 2.

The time course data of tributyrin conversion was fitted to Eq. (6) derived from a Ping Pong Bi Bi mechanism (Supplementary Material B) and the fitted curve is the solid line in Fig. 6a. The accuracy of the model was verified in Supplementary Material F. The experimental

results were found to be consistent with the proposed model, and the regression coefficient ( $R^2$ ) of the fitting was more than 0.99. The  $v_{max}/K_m$  was estimated to be  $7.87 \times 10^{-3} \text{ min}^{-1}$  ( $1.75 \times 10^{-4} \text{ L s}^{-1} \text{ g}^{-1}$  lipase) and  $K_i$  was 28.5 mM. Like most kinetics based on the Ping Pong Bi Bi mechanism presented, only these lumped parameters can be calculated from this fitting with any confidence, therefore no specific rate and equilibrium constants such as  $v_{max}$  and  $K_m$  could be determined individually. Consequently, there is only a limited set of lumped kinetic parameters these results can be compared to. One such study reported that the  $v_{max}/K_m$  was assessed to be 3.44 to  $9.06 \times 10^{-4} \text{ L s}^{-1} \text{ g}^{-1}$  lipase and 5.71 to  $6.85 \times 10^{-6} \text{ L s}^{-1} \text{ g}^{-1}$  lipase in a transesterification reaction catalysed by lipase PS SD and lipase PS IM respectively [44]. Therefore, the Ping Pong Bi Bi kinetics fitted here are reasonable in comparison, and combined with the goodness of fit it can be concluded a Ping Pong Bi Bi mechanism, as the multistep process mentioned above, can be used to describe the triglyceride hydrolysis reactions catalysed by lipase in the SCDR. This indicates that the process intensification by the SCDR enhances the overall reaction rate through the mass transfer discussed previously and does not change the reaction mechanism.

It should be noted that the lipase used in this study has been demonstrated to cleave the 1(3)-position ester bond 8.3 times as fast as the 2-position ester bond [45]. Therefore, most of the produced monobutylin would have existed in the form of 2-monobutylin. Although 2-monobutylin is fairly soluble in water, it is slower for the lipase to hydrolyze. Therefore in this system, the reaction in the water phase was assumed to be negligible and so not taken into account. Therefore separate aqueous phase kinetics was not needed and so not derived.

*3.6. Evaluating more industrially relevant feedstocks: application of the SCDR to vegetable oil hydrolysis*



As discussed above, the SCDR was more effective than a conventional BSTR in tributyrin emulsion hydrolysis, indicating it is a very promising bioreactor for enzyme process intensification. To further demonstrate how the SCDR performs under more industrially relevant conditions, the SCDR was used for the hydrolysis of some kitchen grade vegetable oils: canola oil, soybean oil, sunflower oil and olive oil. Fig. 8 presents the amount of fatty acids produced for the different oils hydrolyzed in the SCDR. For all the oils, the reaction proceeded very fast within the first hour and with a subsequent slowing of the rate, showing the same two step reaction pathway as the tributyrin. After 250 min, a higher fatty acid concentration of 27.7 mM was observed for canola oil compared to 18.5 mM, 20.6 mM, and 23.1 mM for soybean oil, sunflower oil and olive oil respectively. The difference in the lipase catalyzed hydrolysis of the different oils can be attributed to the differences in physical properties and oil impurities between the different tested oils, where previous studies have shown that the oil with the highest content of unsaturated fatty acids produces the highest hydrolysis rate [46, 47]. This trend is reflected in the results here, since canola oil displayed the highest reaction rate and also had the highest amount of unsaturated fatty acids (92.7%), whilst the other three oils have similar unsaturated fatty acid contents (from 84.7% to 87.5%).

Most importantly, the oil hydrolysis obtained in the SCDR is significantly higher than other reactor types reported in the literature. For example, Sachan et al. [10] studied olive oil hydrolysis using lipase in a carbon membrane reactor and obtained fatty acid concentrations of 200 to 250  $\mu\text{mol L}^{-1}$  after 160 min with the same concentration of 25% (v/v) used in this study. Chen et al. [48] investigated a 25% (v/v) olive oil hydrolysis in a cellulose fiber membrane with immobilized lipase and obtained fatty acid concentrations of 1100  $\mu\text{mol}$  after around 400 min with total reactant volume of 240 mL. Compared to these results, the hydrolysis reaction rate was much higher in the SCDR, further indicating intensification of the reaction has most likely occurred. Overall, this result confirms the robustness of the

SCDR for industrially relevant feeds and further indicates that the SCDR is a good candidate for development of industrial scale immobilized enzymatic reactions and reactors.

#### 4. Conclusions

In this study, an innovative SCDR, consisting of a spinning disc with immobilized lipase on woolen cloth has been developed and characterized using tributyrin emulsion hydrolysis as a model reaction. Both the conversion and reaction rate were improved in SCDR compared to that in a conventional BSTR, indicating the reaction intensification has occurred. Reaction intensification is thought to occur through a combination of enhanced mass transfer and mixing, increased interfacial surface area (due to the oil droplet size being decreased due to the sieving action of the wool), the wool protecting the enzymes from shear based deactivation and increased residence time of the substrate in the centrifugally intensified reaction zone due to the liquid hold-up in the wool. Conversion increased by approximately 7% on average as the flow rate increased from 2 to 5 mL s<sup>-1</sup> and the highest conversion occurred at 400 rpm, indicating that a relatively low disc speed is more favorable when the SCDR is applied to enzyme reactions. This is because the loss of enzyme from the spinning woolen support was observed to increase as the surface shear increased, and this phenomenon was more evident after the surface shear reached around 9,500 s<sup>-1</sup>. This is considered to be the ‘critical shear’ below which this SCDR should be operated. The immobilized lipase on woolen cloth was robust to repeated use in the SCDR and 80% of the original activity was maintained after 15 continuous runs. The Ping Pong Bi Bi mechanism was shown to explain the kinetics well, giving a lumped reaction constant ( $v_{max}/K_m$ ) of 7.87×10<sup>-3</sup> min<sup>-1</sup>. Finally, the SCDR was successfully applied in the hydrolysis of different vegetable oils at reaction rates higher than other reactors in the literature.

Overall, the above results indicate that the SCDR is an innovative, superior and robust technology for enhancing enzyme reactions, taking enzyme reactors beyond the current state-of-the-art. While this study focused on lipase-catalyzed oil hydrolysis, the SCDR concept can readily be extended to other enzyme-catalyzed reactions, where process intensification through enhanced mass transfer (and interfacial area) can help increase reaction rate and yield, whilst helping protect the enzymes from detachment and deactivation.

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## 1 **Figure Legends**

2 Figure 1. (a) Schematic diagram of the enzymatic reactor system with the SCDR. (b) Photo of  
3 the enzyme reactor system used, showing the key components. (c) Top view of a woolen  
4 cloth with immobilized lipase on the disc of the SCDR.

5 Figure 2. Time course reaction data from the pH stat comparing tributyrin emulsion  
6 hydrolysis in the BSTR and SCDR at different concentrations: (a) 33 mM, (b) 66 mM, (c) 99  
7 mM, (d) 132 mM; (e) reaction rates at a reaction time of 30 min in the SCDR and BSTR; (f)  
8 reaction rates at a reaction time of 180 min in the SCDR and BSTR. Results are the average  
9 from triplicate measurements and the relative standard deviation <5%.

10 Figure 3. Tributyrin emulsion droplet size versus circulation time in SCDR with/without  
11 cloth. Flow rate: 5 mL s<sup>-1</sup>, spinning speed: 450 rpm. Results were from triplicate  
12 measurements and error bars are the average ± one standard deviation.

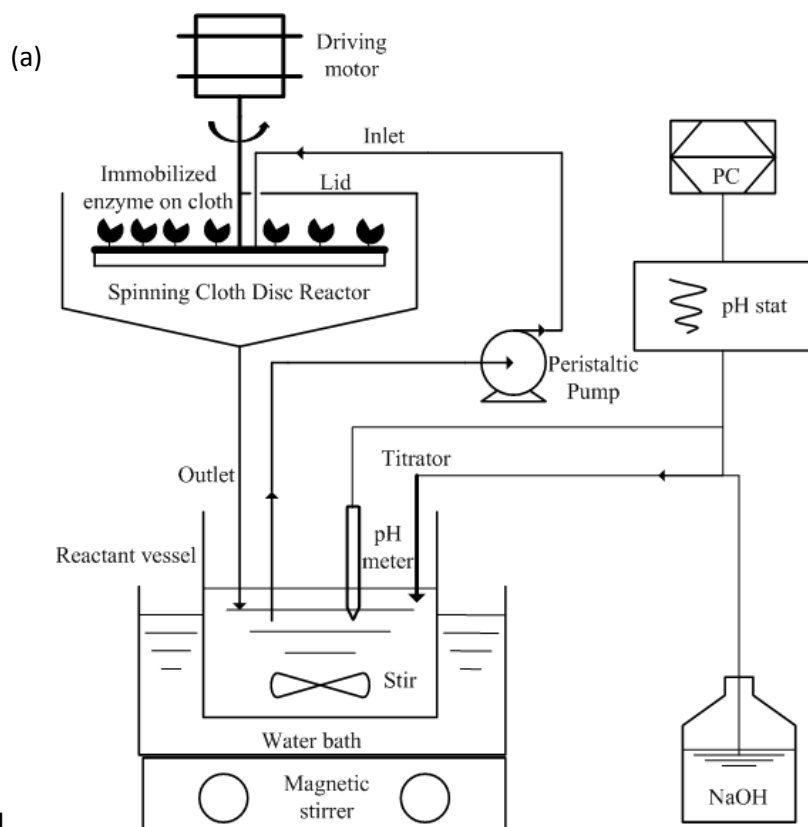
13 Figure 4. Effect of disc speed and flow rate on tributyrin conversion. Substrate concentration:  
14 33 mM, feed volume: 1L, reaction time: 4 h. Half of the experimental data points were from  
15 triplicate measurements, and error bars are the average ± one standard deviation.

16 Figure 5. Leakage of enzyme from the spinning cloth as a function of surface shear. Half of  
17 the experimental data points were from triplicate measurements, and error bars are the  
18 average ± one standard deviation.

19 Figure 6. Activity loss and enzyme leakage of continuous operation of SCDR. Reaction  
20 temperature: 45 °C; Reaction time: 4 h; Flow rate: 5 mL s<sup>-1</sup>; Spinning speed: 350 rpm.  
21 Results were from triplicate measurements and error bars are the average ± one standard  
22 deviation.

Figure 7. (a) Time course reaction data from the pH stat of tributyrin emulsion hydrolysis in SCDR with different concentrations (33, 66, 99, 132 mM) at the same conditions. The scattered points are the continuous experimental data from the pH stat, while the solid lines are calculated from the Ping Pong Bi Bi kinetic model (equations 4 to 6). (b) Progress rate versus concentration of tributyrin emulsion hydrolysis in SCDR with different concentrations.

Figure 8. Time course reaction data from the pH stat of the hydrolysis of different oils in the SCDR: canola oil, olive oil, sunflower oil and soybean oil. Substrate: 25% oil (v/v) (triglyceride concentration is approximately 261.4 mM) and 3% Triton X-100 (v/v) in pH 7 phosphate buffer (0.1 M). Reactant volume: 1 L. Reaction temperature: 45 °C; flow rate: 3 mL s<sup>-1</sup>; spinning speed: 350 rpm. Results are the average from triplicate measurements and the relative standard deviation <5%.



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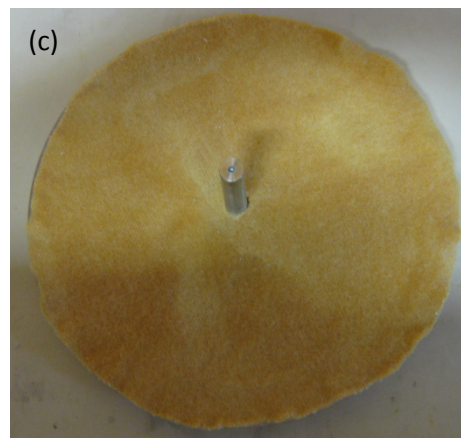
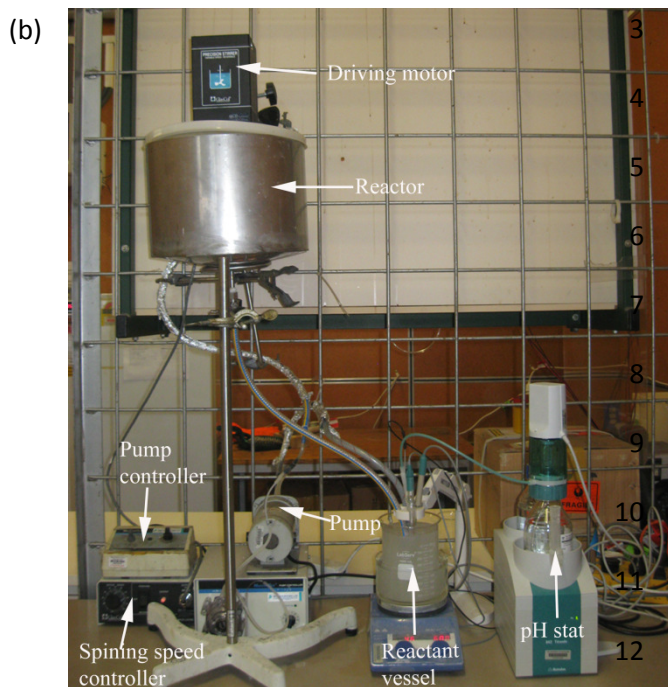


Figure 1

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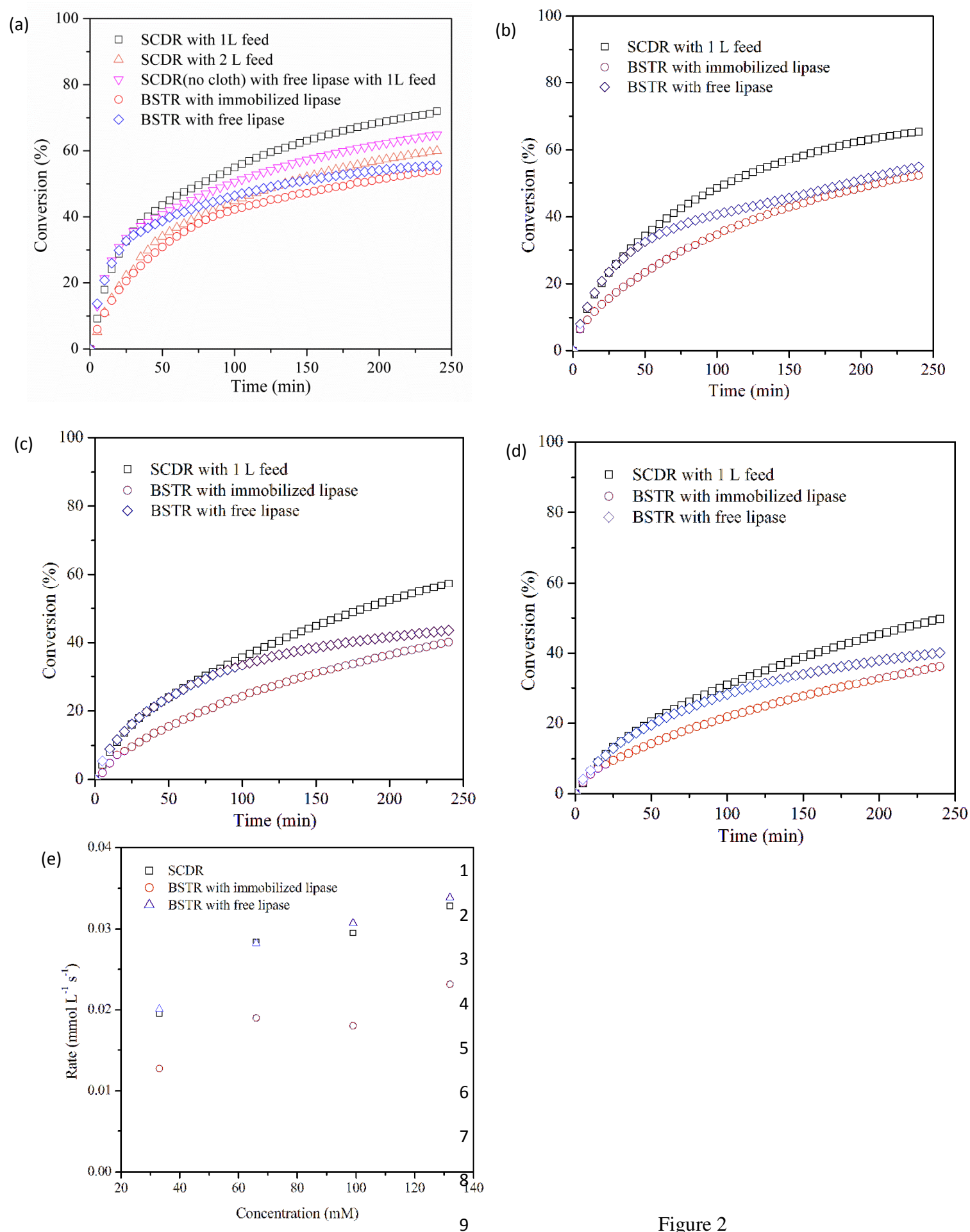
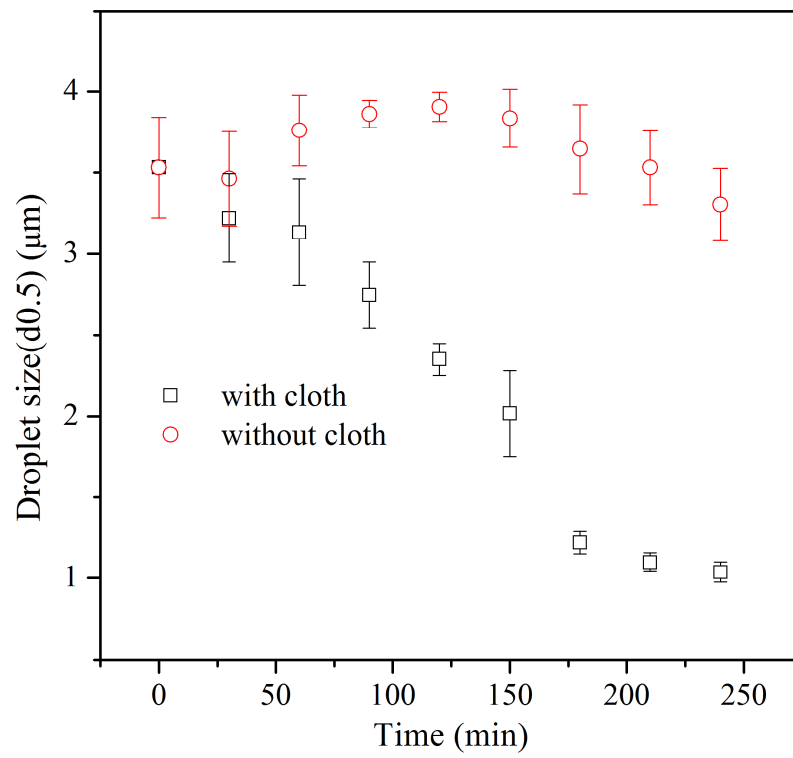


Figure 2

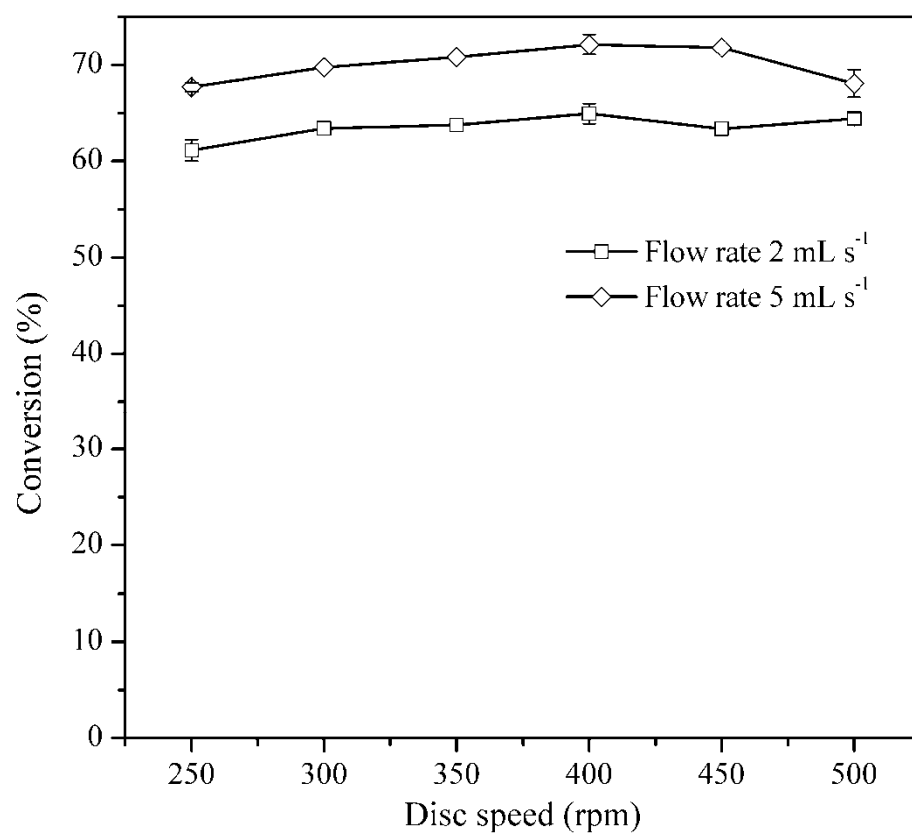


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2 Figure 3

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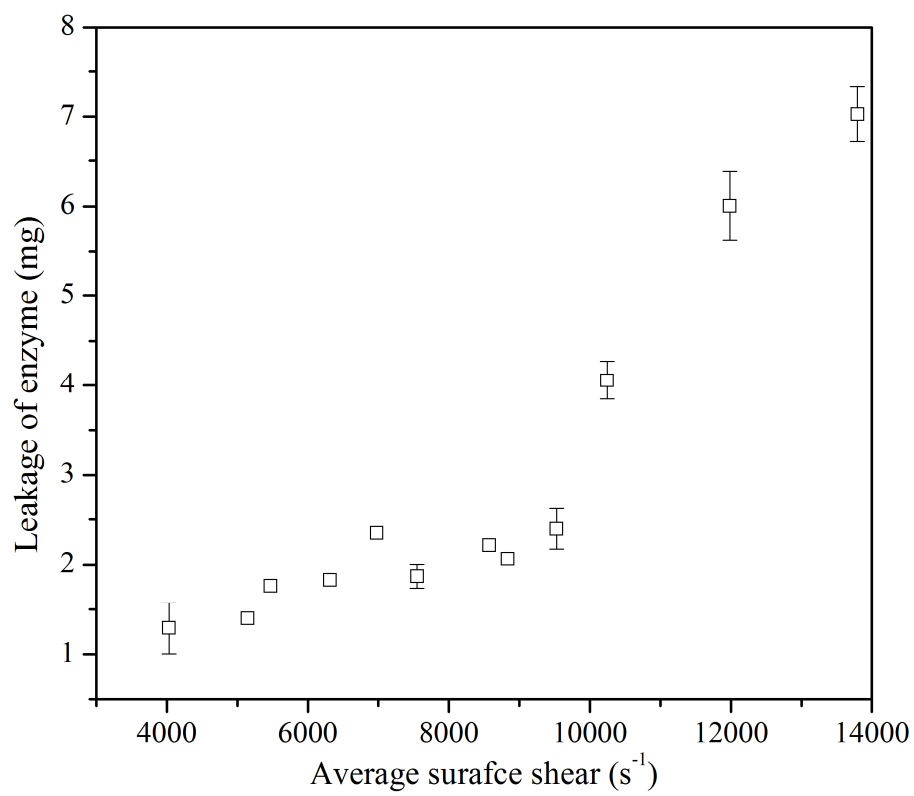
3 Figure 4

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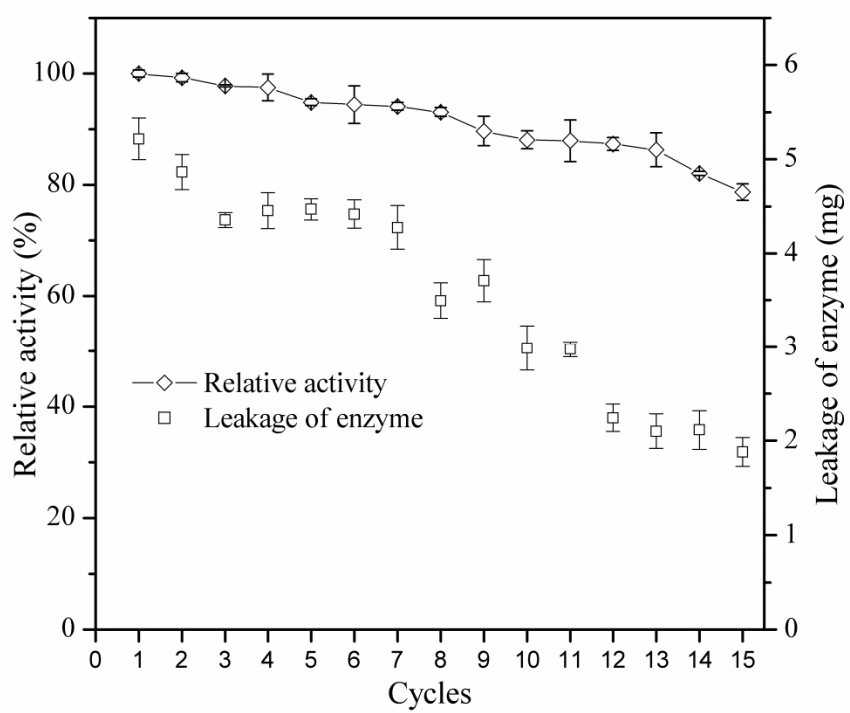


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4 Figure 5

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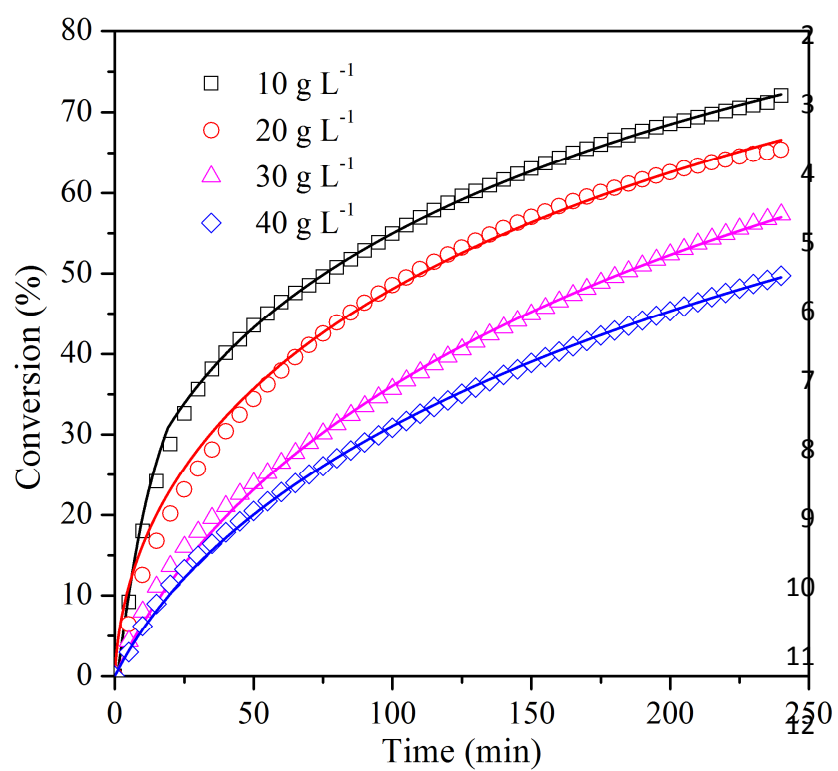
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3 Figure 6

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14 Figure 7a

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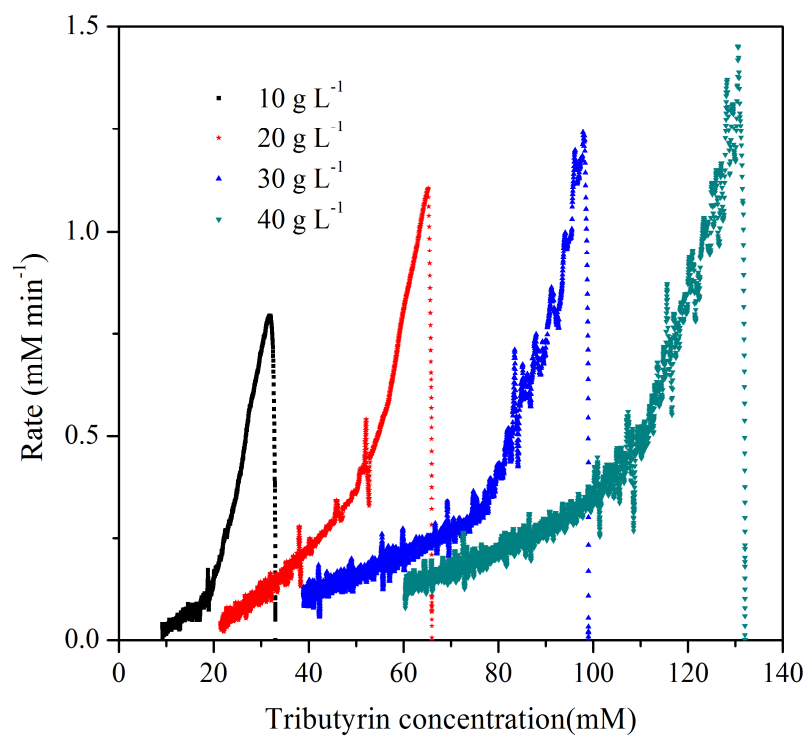
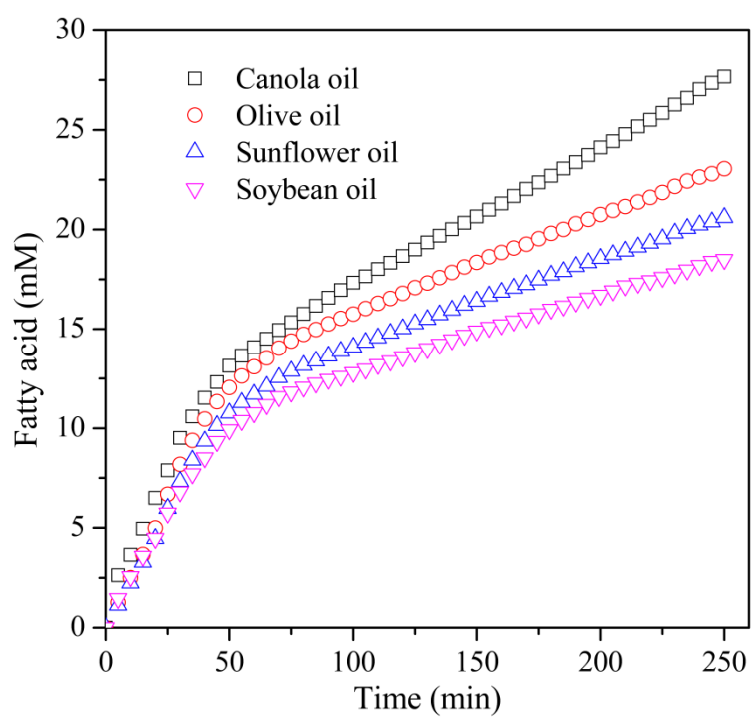


Figure 7b

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3 Figure 8

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